# Postprint: Differences in Physiological Characteristics of Leaf Senescence During the Coloration Period Between Taxodium ascendens and Taxodium distichum

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## Abstract

Taxodium ascendens and Taxodium distichum are common landscape color-leaf tree species whose leaves gradually turn orange (red) during the autumn-winter color transition period. Analyzing the physiological characteristics of leaves during the color transition period in these two Taxodium species is crucial for understanding the process of senescence and color change, and can provide guidance for future applications of landscape color-leaf tree species. This study measured pigment content, antioxidant enzyme activity, osmotic adjustment substance content, malondialdehyde (MDA) content, and chlorophyll fluorescence parameters in senescent leaves of T. ascendens and T. distichum during the color transition period. Combined with statistical methods including significant difference analysis, correlation analysis, linear fitting, and membership function analysis, the study comprehensively evaluated the physiological status of senescent leaves during the color transition period and explored the physiological differences in leaf senescence between the two Taxodium species. The results showed: (1) The AC/CHLa+b ratio of leaf pigments in T. ascendens and T. distichum increased rapidly with senescence, reaching maximum values of 11.46 and 7.13, respectively, which was the direct cause of the orange (red) leaf color. (2) During senescence, the activities of superoxide dismutase (SOD) and catalase (CAT) and the content of soluble sugars (SS) in leaves of both species decreased; MDA content first increased and then decreased; however, the activities of peroxidase (POD), ascorbate peroxidase (APX), and phenylalanine ammonia-lyase (PAL) increased, representing a resistance effect against senescence. (3) Photochemical conversion efficiency and heat dissipation capacity could effectively represent changes in physiological and biochemical indicators and reflect the senescence process. (4) At the same time point, the comprehensive score of T.

ascendens was lower than that of T. distichum, indicating that T. distichum had a better physiological status. In conclusion, the leaf senescence processes of T. ascendens and T. distichum share similar physiological characteristics, but T. ascendens enters senescence and color change earlier. In landscape applications, T. ascendens and T. distichum can be planted in combination to extend the overall color-leaf viewing period.

## Full Text

## Preamble

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### Difference of Physiological Characteristics of Leaf Senescence During Color Transition Period Between *Taxodium ascendens* and *T. distichum*

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**Abstract:** During the autumn and winter seasons, both *Taxodium ascendens* and *T. distichum* undergo a color transition period in which their leaves gradually turn orange or red, making them common ornamental trees with colored foliage in gardens. Analyzing the physiological characteristics of leaf color transition in these two species is crucial for understanding the aging and coloring process of these trees, providing valuable guidance for the application of ornamental trees with colored leaves in garden landscapes. This study conducted measurements on pigment content, antioxidant enzyme activity, osmotic regulation substance levels, malondialdehyde (MDA) content, and chlorophyll fluorescence parameters in aging leaves of *T. ascendens* and *T. distichum* during the color transition period. Statistical methods such as significant difference analysis, correlation analysis, linear fitting, and membership function analysis were employed to comprehensively evaluate the physiological state of aging leaves from both species. The results were as follows: (1) The value of AC/CHLa+b—a pigment ratio present in *T. ascendens* and *T. distichum* leaves—increases rapidly with age, reaching 11.46 and 7.13 respectively; this directly contributes to the orange-red hue observed in their foliage. (2) During aging processes, superoxide dismutase (SOD) and catalase (CAT) activities and soluble sugar content decreased while MDA content initially increased before decreasing again; however, peroxidase (POD), ascorbate peroxidase (APX), and phenylalanine aminolyase (PAL) activities increased, indicating a resistance effect against leaf senescence. (3) The photochemical conversion efficiency and heat dissipation ability can represent the change of physiological and biochemical indexes well, and reflect the aging process. (4) Comprehensive scores show that at any given time *T. ascendens* consistently exhibited lower scores than *T. distichum*, suggesting superior physiological status for *T. distichum*. In conclusion, it is evident that both *T. ascendens* and *T. distichum* demonstrate similar physiological characteristics of leaf senescence, but *T. ascendens* entered into this phase earlier than *T. distichum*. In landscape applications, combining plantings of *T. ascendens* alongside *T. distichum* can extend overall periods for colorful foliage display.

**Keywords:** *Taxodium ascendens*, *Taxodium distichum*, autumn leaves, chlorophyll fluorescence, indicators of aging

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Leaf color constitutes the most direct element of seasonal landscapes. With further development of urban greening in China, numerous colored-leaf tree species have been extensively planted, enriching the visual effects and layering of urban landscapes. As temperatures gradually decrease in autumn and winter, leaves of *Taxodium ascendens* and *T. distichum* exhibit a color transition from green to orange or red, representing both a seasonal display and the natural process of leaf senescence during the color transition period. As colored-leaf species, these trees not only beautify urban scenery but also enhance urban vitality. However, excessively rapid senescence causes leaf abscission, thereby shortening the ornamental period and diminishing landscape effectiveness. Anthocyanin (AC), chlorophyll (CHL), and carotenoid (Car) are the primary pigments determining leaf color, with AC imparting red coloration, CHL green, and Car yellow. Research by Yang (2012) demonstrated that pigment types and ratios directly cause leaf color variation: when the AC/CHL ratio increases, leaves tend toward red, and vice versa toward green; high Car content produces yellow hues. Numerous studies have measured pigment content, concluding that the green-to-red transition typically accompanies dramatic pigment changes, as documented in *Acer rubrum* (Gao et al., 2013), *Acer palmatum* (Cai et al., 2015), and *Quercus nuttallii* (Xu, 2015).

Studies have also confirmed that leaf color change is intimately connected with the senescence process, as chlorophyll content declines rapidly with advancing senescence (Amy et al., 2024; Yang et al., 2024). The antioxidant system represents a critical defense line against senescence, with Huang et al. (2024) emphasizing the importance of antioxidant enzymes for maintaining chloroplast homeostasis. Plants mitigate pigment degradation and delay senescence by enhancing antioxidant enzyme activity to promptly scavenge reactive oxygen species (Shi et al., 2019; Wen et al., 2022). Beyond antioxidant enzymes, pigment synthesis enzymes and nutrients also influence leaf coloration. Phenylalanine ammonialyase (PAL) is a key enzyme in the AC synthesis pathway, and its activity determines AC content changes during plant senescence (Zhan et al., 2022). Soluble sugar (SS) and soluble protein (SP) serve as energy substrates, nutrients, and osmotic regulators for synthetic enzymes, directly regulating plant physiological status (Jia et al., 2022) and enhancing physiological activity during senescence (Liu

& Li, 2017; Han et al., 2022). Numerous studies have found that malondialdehyde (MDA) accumulates during leaf senescence (Liu et al., 2022; He et al., 2024; Liu et al., 2024), with excessive MDA content further damaging tissues, accelerating senescence, and altering leaf color. Lü et al. (2022) identified MDA as an excellent indicator of leaf senescence in maize ear leaves, demonstrating that plant physiological status is closely linked to senescence. Chlorophyll fluorescence parameters characterize plant light utilization capacity and can reflect physiological status changes during senescence (Xue et al., 2021).

*Taxodium ascendens* and *T. distichum* are both large deciduous trees in the family Taxodiaceae, genus *Taxodium*, with extremely strong water tolerance and the ability to grow normally in water, making them commonly used species in Chinese landscape design. Current domestic and international research on these species has focused on nutrient characteristics (Ding et al., 2021), community succession (Middleton et al., 2021), stress responses (He et al., 2019; He et al., 2021), and genetic diversity (Ikezaki et al., 2016). *Taxodium ascendens* and *T. distichum* are widely planted in central China, becoming essential autumn and winter color-display trees in cities such as Hangzhou, Wuhan, and Nanjing. In contrast, their landscape application in southern China is less prominent. Moreover, no studies have analyzed the senescence characteristics of these two species during the color transition period in southern China, particularly regarding physiological differences in the leaf senescence process. Therefore, this study utilized *T. ascendens* and *T. distichum* from the Nanning region as experimental materials, measuring indicators including pigment content, enzyme activity, osmotic regulation substances, and chlorophyll fluorescence parameters during the color transition period. By comparing and analyzing the physiological processes of leaf senescence in the two species, we aimed to address the following questions: (1) What are the senescence characteristics of *T. ascendens* and *T. distichum* leaves during the color transition period? (2) What are the physiological differences in the leaf senescence process between the two species? This research seeks to provide a scientific foundation for further understanding the color transition mechanisms in senescing leaves of these species and for their landscape application.

### 1.1 Study Site Overview

The experimental site was located at the nursery of Guangxi University Forestry College (108°17 9.00 E, 22°50 28.41 N), characterized by a subtropical monsoon climate with an average annual precipitation of 1310 mm, mean annual temperature of 21.6°C, and average relative humidity of 80%. The site features abundant rainfall, flat terrain, and long sunshine duration.

### 1.2 Experimental Materials

The experimental materials consisted of healthy 2-year-old seedlings of *Taxodium ascendens* and *T. distichum*. The containers were plastic pots measuring 12 cm in height and 10 cm in diameter, filled with coconut coir as substrate, with one plant per pot. Fifty pots were prepared for each species, with each pot serving as one biological replicate, totaling 100 pots. Watering and pest management followed conventional practices.

### 1.3 Experimental Methods

During the color transition period, leaves of *T. ascendens* and *T. distichum* gradually senesced, displaying a transition from green to orange or red, with individual plants bearing leaves of different colors. On the morning of December 30, 2021, under clear weather conditions, leaves at different color transition stages were collected from seedlings of both species, corresponding to three color stages: green (Stage 1), green-orange (Stage 2), and orange/red (Stage 3) [FIGURE:1]. Samples were immediately placed in ziplock bags, stored in an ice box, and rapidly transported to the laboratory for determination of pigment content and physiological-biochemical indicators. Seedlings were brought to the laboratory and placed in darkness for dark adaptation; chlorophyll fluorescence parameters were measured after 20 minutes, with three replicates per measurement.

### 1.4 Indicator Measurements

Pigment indicators included chlorophyll a (CHLa), chlorophyll b (CHLb), total chlorophyll (CHLa+b), carotenoids (Car), and anthocyanins (AC). Physiological-biochemical indicators comprised superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), phenylalanine ammonialyase (PAL), soluble protein (SP), and soluble sugar (SS).

AC content was determined following the method of Wrolstad et al. (2020), PAL activity was measured according to Wang et al. (2004), and CHLa, CHLb, CHLa+b, Car, SOD, POD, CAT, APX, SP, and SS were assayed following the protocols in *Experimental Supervision of Plant Physiology* (Gao, 2006). Chlorophyll fluorescence indicators were measured using a PAM-2500 fluorometer: maximum photosynthetic efficiency (Fv/Fm), actual photosynthetic efficiency (Y(II)), quantum yield of non-regulated energy dissipation (Y(NO)), and non-photochemical quenching coefficient (NPQ). All parameters were measured with three replicates.

### 1.5 Data Analysis

DPS 7.05 software was used for variance analysis and multiple comparison tests (Duncan's method, $P < 0.05$). Origin 2019 software was employed for linear fitting, correlation analysis, and graphing. To investigate the overall physiological status of *T. ascendens* and *T. distichum* leaves at different stages, the membership function method (Xu et al., 2009) was applied to calculate average membership degrees and generate comprehensive rankings. Related calculations were performed using Excel 2019 software.

## 2.1 Leaf Pigment Content and Ratios in *T. ascendens* and *T. distichum*

As shown in [FIGURE:2]A–D, *T. distichum* exhibited higher CHL content than *T. ascendens* at all stages, but lower AC/CHLa+b ratios. CHL and Car contents in both species decreased significantly with senescence. Conversely, AC content increased, reaching its maximum in Stage 3 at 1.91 $mg \cdot g^{-1}$ in *T. ascendens* and 2.17 $mg \cdot g^{-1}$ in *T. distichum* [FIGURE:2]E. The AC/CHLa+b ratio increased with senescence in both species, with leaf color gradually turning red [FIGURE:2]F. *Taxodium ascendens* maintained higher AC/CHLa+b values than *T. distichum* at all stages.

As illustrated in

A, the relative AC proportion in *T. ascendens* increased from 80% (Stage 1) to 91% (Stage 3), while CHLa+b and Car contents decreased from 18% and 2% (Stage 1) to 8% and 1% (Stage 3), respectively. In *T. distichum*

B, the relative AC proportion rose from 63% (Stage 1) to 87% (Stage 3), with CHLa+b and Car contents declining from 33% and 4% to 12% and 1% (Stage 3), respectively. *Taxodium ascendens* showed higher AC content but lower CHLa+b content than *T. distichum* at all stages. Since the AC/CHLa+b ratio determines leaf color presentation, these results indicate that *T. ascendens* leaves undergo color change earlier than those of *T. distichum*.

## 2.2 Physiological and Biochemical Indicators in *T. ascendens* and *T. distichum*

As depicted in [FIGURE:4]A–D, SOD and CAT activities in both species gradually declined with senescence, reaching their lowest values in Stage 3. APX activity showed the opposite trend, peaking in Stage 3 at 1013.34 and 1193.33 $U \cdot g \cdot FW^{-1}$, respectively. POD activity initially increased then decreased, with maximum values of 1720 and 1536 $U \cdot g \cdot FW^{-1}$ observed in Stage 2. As shown in [FIGURE:4]E, PAL activity increased with senescence in both species, reaching its highest values in Stage 3 at 3824 and 5286.67 $U \cdot g \cdot FW^{-1}$, respectively, though *T. ascendens* consistently exhibited lower PAL activity than *T. distichum*. MDA content in both species first decreased then increased, peaking in Stage 3 at 4.24 and 5.75 $mg \cdot g^{-1}$, respectively [FIGURE:4]F.

As illustrated in

A, SP content in *T. distichum* initially increased then remained stable, rising from 8.26 $mg \cdot g^{-1}$ and stabilizing at approximately 9.58 $mg \cdot g^{-1}$. In contrast, SP content in *T. ascendens* first decreased then increased, declining from 7.75 $mg \cdot g^{-1}$ to 5.66 $mg \cdot g^{-1}$ before rising to 8.55 $mg \cdot g^{-1}$, with *T. ascendens* showing lower SP content than *T. distichum* at all stages. As shown in

B, SS content in both species gradually decreased with senescence, dropping from 23.00% and 29.75% to 14.15% and 14.99%, respectively, with *T. ascendens*

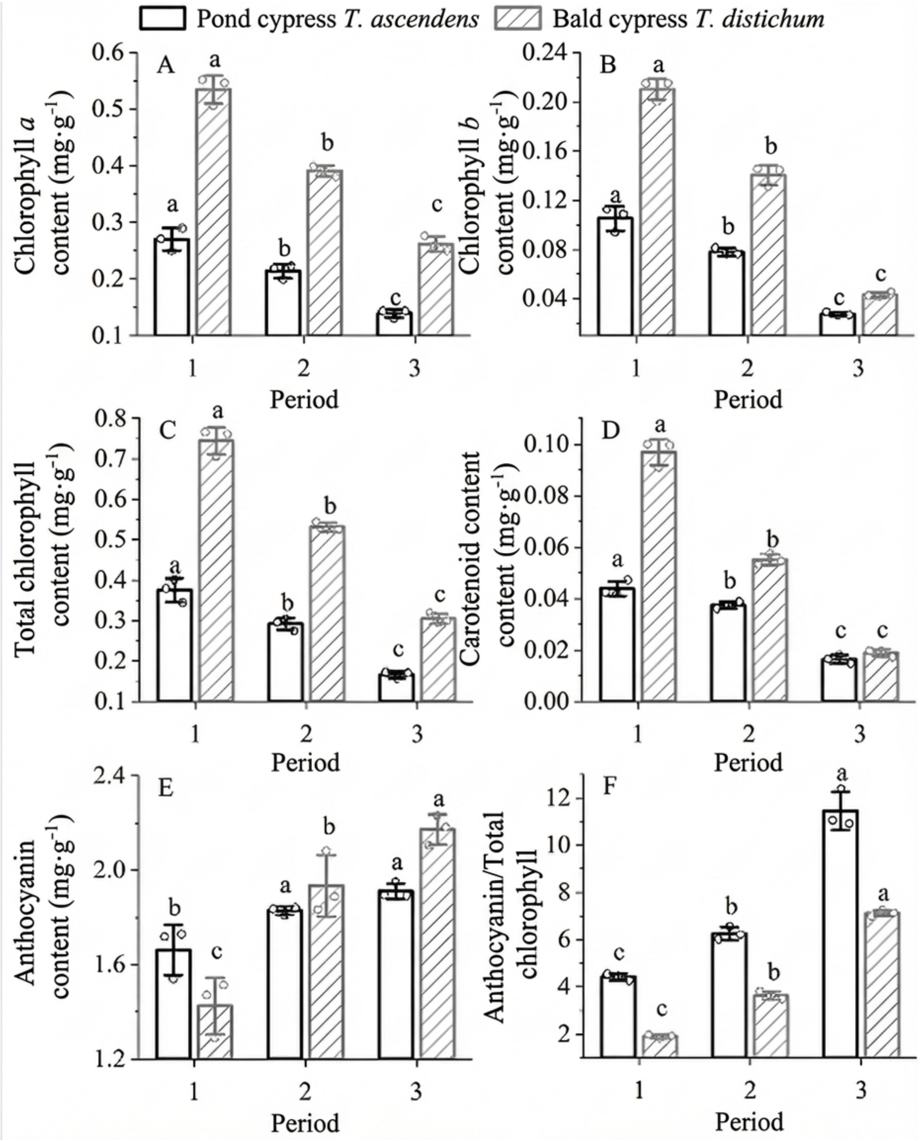

Figure 1: Figure 3

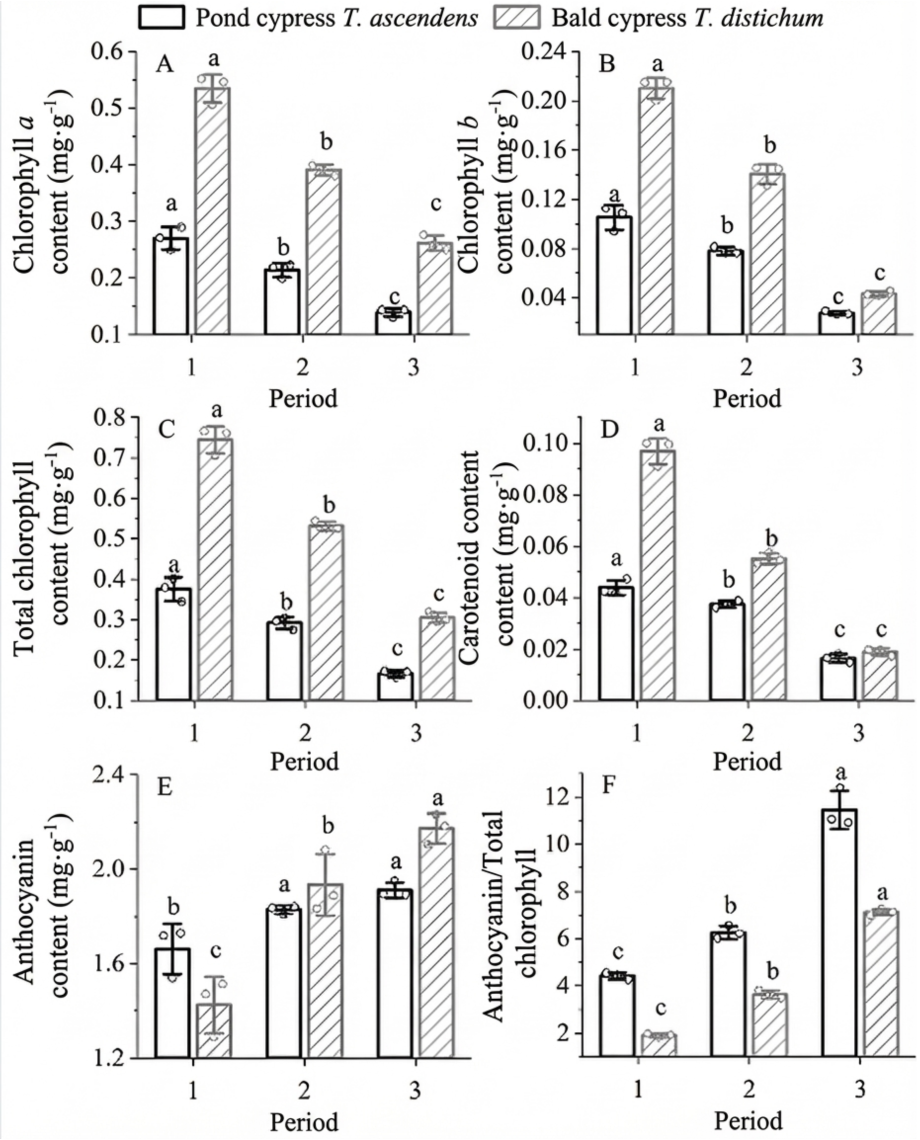

Figure 2: Figure 3

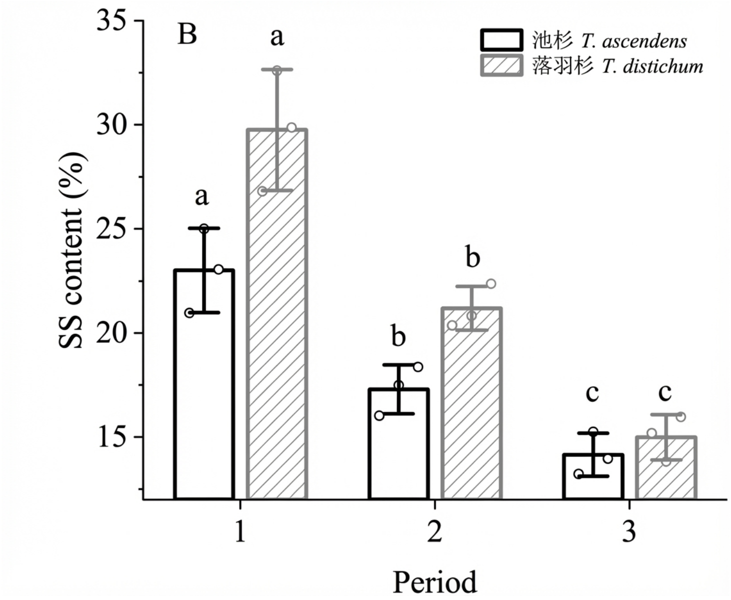

Figure 3: Figure 5

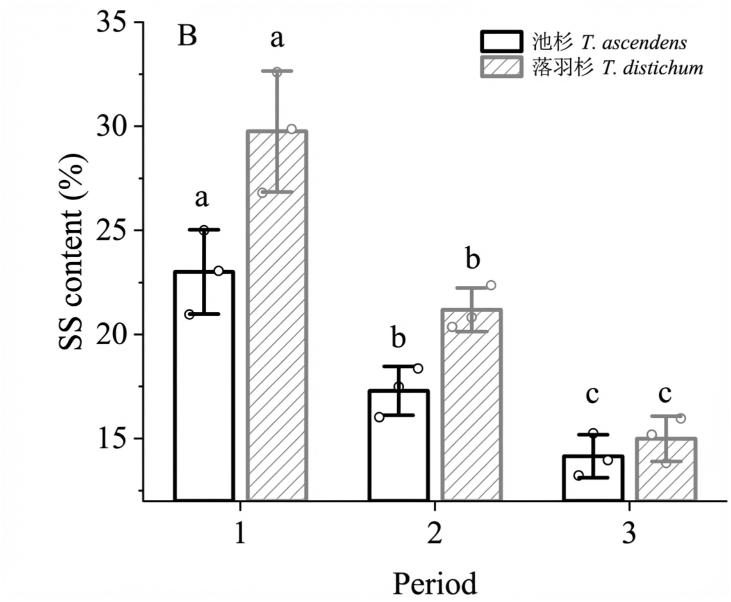

Figure 4: Figure 5

maintaining lower SS content than *T. distichum* throughout.

These findings demonstrate that physiological and biochemical indicators changed significantly across stages in both species. *Taxodium distichum* exhibited higher antioxidant enzyme activity and MDA content than *T. ascendens*, which may represent an important factor contributing to the earlier senescence and color transition observed in *T. ascendens*.

Chlorophyll fluorescence parameters reflect plant light utilization capacity. Fv/Fm represents the efficiency with which Photosystem II (PSII) converts absorbed light energy into chemical energy, while Y(II) indicates actual light energy conversion efficiency under suitable illumination. Heat dissipation reduces damage to photosynthetic apparatus, and NPQ reflects changes in heat dissipation capacity, serving as an important photoprotection indicator. Y(NO) represents the quantum yield of non-regulated energy dissipation in the PSII system, an important photodamage indicator; elevated Y(NO) values suggest that photochemical conversion efficiency and protective regulatory mechanisms such as heat dissipation are insufficient to consume absorbed photochemical energy, potentially resulting in light-induced damage.

As shown in

A–D, Fv/Fm in both species gradually decreased during leaf senescence, dropping to 0.27 and 0.30 in Stage 3, respectively. Y(II) also declined progressively, decreasing to 0.55 and 0.45. Conversely, Y(NO) increased gradually, rising to 0.46 and 0.55 in Stage 3, with *T. ascendens* showing lower Y(NO) values than *T. distichum* at all stages. NPQ also increased progressively, reaching 0.98 and 0.73 in Stage 3, respectively, with *T. distichum* exhibiting lower NPQ values than *T. ascendens* at all stages. These results indicate that despite advancing senescence, *T. ascendens* maintained higher actual light energy conversion efficiency and stronger heat dissipation capacity (as evidenced by lower photodamage indicator Y(NO)) than *T. distichum*, suggesting that *T. ascendens* possesses a superior photodamage response capacity overall.

## 2.3 Correlation Analysis of Physiological and Biochemical Indicators

As shown in

A,B, numerous significant correlations existed among physiological and biochemical indicators in both species. CHL, Car, Fv/Fm, and Y(II) were all significantly positively correlated with SOD, CAT, and SS, but significantly negatively correlated with APX and PAL. Conversely, AC, AC/CHLa+b, Y(NO), and NPQ were significantly negatively correlated with SOD, CAT, and SS, but significantly positively correlated with APX and PAL. The consistent decreasing trends of SOD, CAT, and SS with CHL, Car, Fv/Fm, and Y(II) during senescence suggest that reduced SOD and CAT activities may lead to reactive oxygen species accumulation, inhibiting photochemical processes and conversion

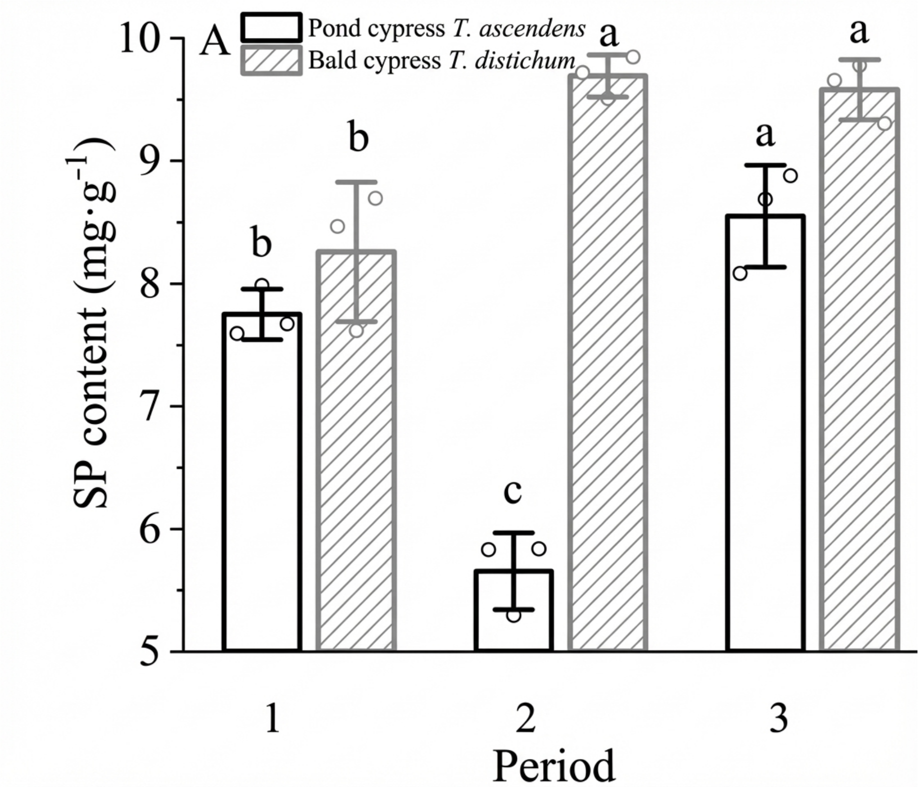

Figure 5: Figure 6

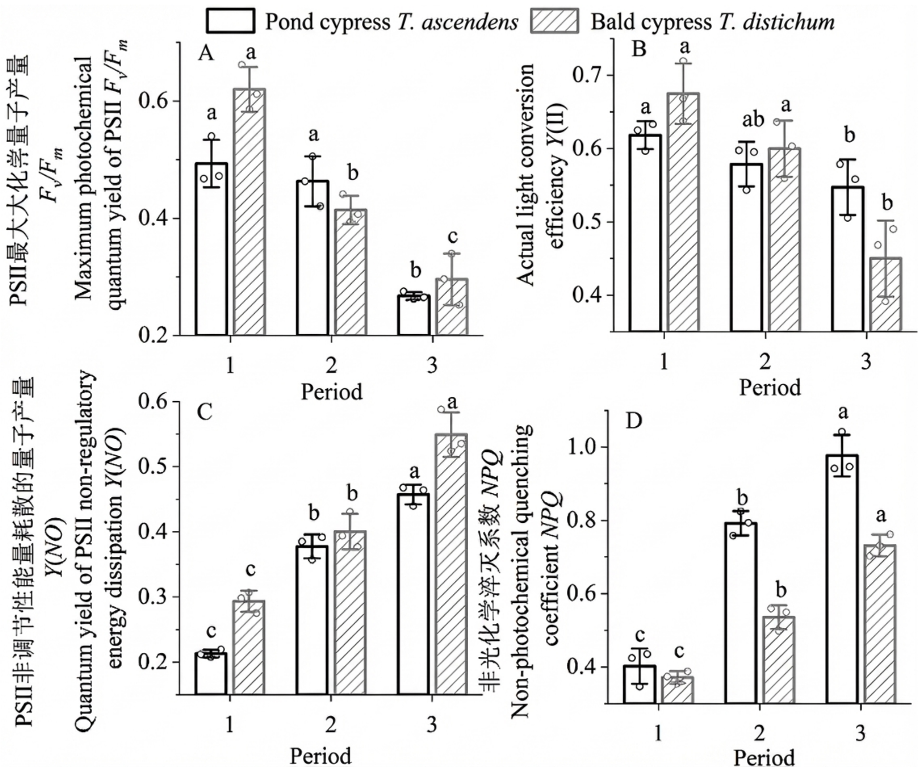

Figure 6: Figure 7

efficiency. Additionally, declining SS content fails to provide energy for SOD and CAT synthesis, causing further activity reduction, and cannot maintain osmotic potential, resulting in additional MDA accumulation.

In contrast, APX activity increased consistently with Y(NO) and NPQ, supplementing SOD and CAT deficiencies to maintain chloroplast physiological homeostasis and preserve heat dissipation capacity, thereby reducing photochemical damage. Furthermore, elevated PAL activity promoted AC synthesis, assisting in photoprotection. These findings demonstrate synergistic interactions among antioxidant enzymes, osmotic regulation substances, and chlorophyll fluorescence parameters during leaf senescence in both species. The essentially identical correlation patterns between *T. ascendens* and *T. distichum* suggest that the two species employ similar physiological mechanisms underlying leaf color change.

## 2.4 Linear Fitting of Physiological and Biochemical Indicators

During the leaf color transition period in *T. ascendens* and *T. distichum*, significant changes in physiological and biochemical indicators reflected the progressive senescence process. To investigate linear relationships between chlorophyll fluorescence parameters and leaf color, enzyme activity, and osmotic regulation substances during senescence, AC/CHLa+b was selected as the leaf color index, and SOD, CAT, APX, PAL, and SS—indicators showing significant correlations with chlorophyll fluorescence parameters in the correlation heatmaps—were chosen for linear fitting.

As shown in [FIGURE:8], all fitting relationships were significant ($P < 0.05$) except for the fit between Y(II) and PAL in *T. ascendens* ($P = 0.61$). The best-fitting relationships were NPQ vs. AC/CHLa+b in *T. distichum* ($R^2 = 0.95$), Y(NO) vs. PAL in *T. distichum* ($R^2 = 0.95$), Y(NO) vs. APX in *T. distichum* ($R^2 = 0.94$), and Y(NO) vs. AC/CHLa+b in *T. distichum* ($R^2 = 0.93$). Notably, many fitting groups showed lower $R^2$ values for *T. ascendens* than for *T. distichum*, indicating poorer fitting stability and reflecting differences in the degree of association among physiological and biochemical indicators between the two species.

In both species, elevated SOD and CAT activities corresponded to increased Fv/Fm and Y(II) values and decreased Y(NO) and NPQ values. The alternating changes between APX activity and SOD/CAT activities may represent a dynamic mechanism for maintaining physiological balance. These results indicate that higher antioxidant enzyme activity can maintain PSII homeostasis, facilitating photochemical conversion, with SS exerting similar effects. However, senescence continued irreversibly; when antioxidant enzyme activity and osmotic regulation substance content could no longer be sustained, both species coped with excess light energy by enhancing heat dissipation capacity to reduce photochemical damage. During this process, PAL activity increased, thereby raising AC content and promoting AC/CHLa+b elevation. Higher AC content also served as an alternative electron sink, compensating for declines in other metabolic processes such as sugar, starch, and protein synthesis, indirectly providing photoprotection and synergistically reducing photochemical damage (Mitsutoshi et al., 2024). Consequently, both AC and AC/CHLa+b showed positive fitting relationships with Y(NO) and NPQ. Overall, photochemical conversion efficiency and heat dissipation capacity effectively represent changes in the selected physiological and biochemical indicators, reflecting the senescence process.

## 2.5 Membership Function Comprehensive Evaluation of Physiological Status

By converting membership degrees for various indicators across different color transition stages in *T. ascendens* and *T. distichum*, average membership degrees were calculated and comprehensive rankings were generated (Table 1). Higher average membership degrees indicate better physiological condition and higher ranking. The results show that comprehensive scores for both species decreased progressively with senescence, indicating deteriorating physiological status. Combined with previous data, Stage 3 showed substantially reduced enzyme activity and chlorophyll content in both species, representing obvious senescence and greater susceptibility to leaf abscission. Furthermore, at the same time point, *T. distichum* consistently achieved higher comprehensive rankings than *T. ascendens*, indicating superior physiological status and later senescence in *T. distichum*, manifested as greener leaf color. This represents an important reason why *T. distichum* changes color and sheds leaves later than *T. ascendens*.

## 3.1 Senescence Characteristics of *T. ascendens* and *T. distichum* Leaves During Color Transition

Leaf color change represents an adaptive mechanism evolved by plants in response to low temperatures (Zhan et al., 2019), with the internal cause being alterations in pigment content and ratio within leaf cells. Plant leaf senescence during low-temperature periods is often accompanied by color changes, implying that senescence induces pigment synthesis or degradation. In this study, CHLa, CHLb, CHLa+b, and Car contents in both *T. ascendens* and *T. distichum* decreased with senescence, while AC content gradually increased, manifesting as the color transition from green to orange or red. These results align with conclusions from Ougham et al. (2008). Notably, in both species, AC content held an absolute advantage at all stages and continued increasing with senescence, while the AC/CHLa+b ratio rose rapidly—representing the direct cause of color change (Wang et al., 2023). Chu et al. (2013) proposed that leaves appear green when chlorophyll content exceeds 60%, red when anthocyanin content ranges from 60% to 80%, and yellow when both chlorophyll and anthocyanin contents fall below 40%. In this study, AC content in both species already exceeded 60%

in Stage 1, suggesting that the leaf senescence program may have already been initiated at this stage.

In contrast, the AC/CHLa+b ratio in *T. ascendens* remained consistently higher than in *T. distichum* at all stages, resulting in more orange-red leaf coloration. Han (2014) found that the AC/CHL ratio in *T. ascendens* was higher than in *T. distichum* at nearly all sampling times, consistent with our findings. These results demonstrate that *T. ascendens* senesces earlier than *T. distichum*, with its colored-leaf landscape appearing sooner. In landscape design, combined planting of *T. ascendens* and *T. distichum* can extend the overall ornamental period. Additionally, Sun (2013) reported that the ornamental period for these species in Hangzhou extends from October to January of the following year, whereas in Nanning the ornamental period only begins in December. This substantial difference likely results from Nanning's higher average temperatures and delayed onset of low temperatures in autumn and winter. In summary, the higher AC/CHLa+b ratio in *T. ascendens* at the same stage indicates earlier entry into the senescence and color transition process, representing an intuitive manifestation of senescence differences between the two species.

## 3.2 Physiological Characteristics of the Senescence Process in *T. ascendens* and *T. distichum* Leaves

Antioxidant enzymes protect against senescence by scavenging reactive oxygen species (ROS) to prevent tissue and membrane lipid damage and maintain cellular morphology and structure. SOD serves as the first line of antioxidant defense, converting superoxide radicals ($O_2^-$) into $H_2O_2$ and $O_2$ through dismutation reactions, after which CAT and POD jointly decompose $H_2O_2$ into $H_2O$ and $O_2$, eliminating ROS toxicity. APX primarily participates in $H_2O_2$ decomposition via the AsA-GSH cycle, effectively scavenging $H_2O_2$ in chloroplasts (Dhriti et al., 2019; Han et al., 2020; Rajput et al., 2021).

Previous research has shown that plant metabolic activity and nutrient levels decline with advancing senescence (Wang et al., 2020), ultimately leading to significantly reduced antioxidant enzyme activity. In this study, SOD and CAT activities decreased with senescence, consistent with these findings. However, POD activity in both species increased initially then decreased, while MDA content first decreased then increased, indicating that POD played a primary role in maintaining physiological status in senescing leaves. Nevertheless, as senescence inevitably progressed, POD activity decreased in Stage 3. This pattern resembles results from Yang (2019) but differs from those of Jiang (2016) and Han (2014), suggesting that such discrepancies may be related to both species-specific senescence characteristics and environmental factors—warranting further comparative studies across different species and regions. Senescence accumulates substantial ROS, destroying chloroplasts and reducing chlorophyll content (Muhmmad et al., 2024). In this study, continuously increasing APX activity in both species indicated their efforts to maintain chloroplast system stability to resist senescence. These findings demonstrate that POD and APX play important roles in scavenging ROS and maintaining physiological homeostasis during senescence in both species.

Furthermore, SP and SS can reduce MDA damage by balancing osmotic potential and provide energy and nutrients for enzyme synthesis, directly participating in plant physiological responses to senescence. In this study, SP content in *T. ascendens* first decreased then increased with senescence, while *T. distichum* SP content first increased then stabilized, suggesting that both species may increase SP content to balance osmotic differences and maintain membrane stability. The decline in SS content in both species indicates consumption during senescence. PAL activity often reflects AC content levels, and AC plays an important role in enhancing antioxidant enzyme activity and protecting against light damage during senescence. In this study, PAL activity in both species increased gradually with senescence, thereby elevating AC content to resist senescence.

During senescence, plant leaf photosynthetic capacity declines dramatically, making repair of light damage caused by chloroplast destruction crucial for maintaining cellular function. Chlorophyll fluorescence reflects plant photosynthetic physiological levels and the capacity to process excess light energy (Oquist & Chow, 1992), thereby directly indicating the degree of light damage in senescing leaves. Junker et al. (2016) found that chlorophyll photochemical reaction capacity gradually diminishes with leaf senescence, reducing overall chlorophyll fluorescence levels. In this experiment, Fv/Fm and Y(II) in both species decreased gradually across stages, while Y(NO) increased, indicating that light energy conversion efficiency declined with senescence and excess light energy accumulated progressively, resulting in light damage that may accelerate leaf senescence. These results align with Danilova et al. (2020). Both species actively resisted senescence, as evidenced by gradually increasing NPQ with leaf senescence, enhancing heat dissipation capacity to process excess light energy and strengthen photoprotection levels, reflecting a self-protection mechanism against light damage. These findings are similar to Guo et al. (2022) and Liu et al. (2022). However, some studies have reported that overall NPQ decreases in *Arabidopsis* during late senescence, with only high NPQ at the base of rosette leaves (Astrid et al., 2004), inconsistent with our results. This discrepancy may arise because chloroplast systems were completely destroyed and nonfunctional in those *Arabidopsis* plants, causing loss of the chlorophyll fluorescence pathway, or because senescence degrees differ among various plant functional parts. Although *T. ascendens* was more senescent than *T. distichum* at the same time point, *T. ascendens* maintained higher actual light energy conversion and heat dissipation capacities, resulting in photodamage response capacity no lower than that of *T. distichum*, which is more conducive to active resistance against senescence.

## 3.3 Comprehensive Evaluation of Senescence Physiological Parameters in *T. ascendens* and *T. distichum*

Numerous studies have demonstrated that changes in pigment and MDA content and antioxidant enzyme activity reflect senescence progression (Li et al., 2004; Kang et al., 2019; Mallesham et al., 2024). Other research indicates that leaf senescence is accompanied by chlorophyll fluorescence kinetic changes (Ayoub et al., 2021; Xue et al., 2021). In this study, correlation analysis revealed synergistic effects among physiological and biochemical indicators in senescing leaves of both species. Building on these conclusions, we performed linear fitting between chlorophyll fluorescence parameters and pigment content, MDA content, and antioxidant enzyme activity to explore the indicative role of chlorophyll fluorescence parameters in senescence processes of the two species. The best-fitting relationships were Y(NO) vs. SOD and NPQ vs. AC/CHLa+b. Domestic and international studies have also utilized chlorophyll fluorescence parameters to fit greenness indices (Liu et al., 2004), leaf area (Yin et al., 2004), and predict senescence timing and evolution (Astrid et al., 2004; Marta et al., 2014). These findings demonstrate that chlorophyll fluorescence parameters are important plant indicators that can be effectively applied to plant status analysis and monitoring.

Membership function comprehensive ranking revealed that *T. distichum* maintained better overall physiological condition and stronger senescence resistance than *T. ascendens* at the same time point, representing an important reason for its later color change and leaf abscission.

During the color transition period, senescing leaves of *T. ascendens* and *T. distichum* transition from green to orange or red, with the AC/CHLa+b ratio increasing rapidly with senescence as the direct cause of leaf color change. Both species exhibit similar physiological characteristics during leaf senescence, primarily resisting senescence through regulation of antioxidant enzymes (mainly POD and APX), osmotic regulation substances (SP and SS), PAL activity, and chlorophyll fluorescence parameters. These mechanisms reduce MDA content, enhance heat dissipation capacity, prevent photodamage caused by excess light energy accumulation, and delay excessive leaf senescence and abscission. However, senescence is irreversible; the rapid increase in MDA content signals the arrival of late senescence, with leaves turning orange or red. Although *T. ascendens* demonstrates superior light energy utilization and photodamage protection compared to *T. distichum*, its overall physiological status is disadvantaged, manifesting as more advanced senescence at the same time point. Photochemical conversion efficiency and heat dissipation capacity effectively represent changes in physiological and biochemical indicators, reflecting the senescence process. For future landscape applications, combined planting of *T. ascendens* and *T. distichum* is recommended to extend the overall colored-leaf ornamental period.

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